



## Niche differentiation and evolution of comammox *Nitrospira* through a comparative genomics analysis

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Niche differentiation and evolution of comammox *Nitrospira* through a comparative genomics analysis.

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Nitrification, the biological oxidation of ammonium to nitrate, is a fundamental process in the nitrogen cycle and plays an important role in natural and engineered systems. Throughout the last century, nitrification was assumed to be a two-step process executed by two different functional groups, ammonia oxidizing prokaryotes (AOP) and nitrite oxidizing bacteria (NOB). Recently, several articles have shown the capability of a single microorganism, belonging to the genus *Nitrospira*, to carry out the complete oxidation of ammonia to nitrate (comammox). *Nitrospira* spp. are widespread in both natural and engineered ecosystems associated with nitrogen cycling and different species are frequently observed to coexist in the same environment. Besides recent discoveries pointing towards versatile metabolism in some *Nitrospira* species, little is known about the functional potential of the two comammox *Nitrospira* clades, and the factors involved in niche-partitioning between comammox and canonical *Nitrospira*.

A comparative genomics analysis was conducted with five genomes recovered from a groundwater-fed rapid sand filter (including both comammox clades and a nitrite-oxidizing *Nitrospira* population genome) and high quality published *Nitrospira* genomes, to reveal distinct genomic features within *Nitrospira*. In addition, we investigated the evolution of the ammonia oxidation pathway in comammox *Nitrospira*. This analysis revealed distinct genetic capabilities of the different comammox clades and canonical *Nitrospira* which can help to explain the coexistence and niche partitioning of *Nitrospira* spp. These divergences range from the nitrogen source utilization capacity to the ability for electron donor versatility, and other characteristics such as stress response. With respect to the evolutionary history of comammox *Nitrospira*, our analysis indicates transfer events with betaproteobacterial ammonia oxidizers. In addition, transfer events between comammox clade A and clade B were also detected for genes belonging to the ammonium oxidation pathway.

Together, these results expand the actual knowledge of the ecology and evolution of the recently discovered comammox *Nitrospira*.